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A Systematic Review of Serological and Clinicopathological Features and Associated Risk Factors of Avian Pox

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Abstract: Avian pox is a viral disease of a wide range of both domestic and wild bird species caused by the virus of genus Avipox virus under the family Poxviridae. There are three main strains of the virus. These are Fowlpox, Pigeonpox and Canarypox .The disease has two forms. The dry or cutaneous form is mainly characterized by skin lesions on the unfeathered parts of the bird's body. This form of the disease has high prevalence but less severity. The other form is diphtheritic or wet form which is characterized by lesions in the mouth and upper respiratory tract. The disease is widely distributed worldwide. The disease affects birds irrespective of differences in sex, age and breed. The virus enters into the body of birds through abraded skin or bite of mosquitoes. Contaminated environment, carrier birds and mosquitoes are sources of infection. The virus can survive for long period of time in the environment. There are some factors for increase in the incidence of the disease like breed differences, Managemental practices and environmental conditions. Weakness, emaciation, difficulty in swallowing and breathing, vision problems, a reduction in egg production, soiled facial feathers, conjunctivitis and edema of the eyelids and the presence of the characteristic wart-like growths are the general clinical signs of the disease. Secondary complications are common as a result of break in the continuity of the skin caused by pox lesions. Clinical signs, gross lesions, serological tests, electron microscopy and molecular methods to identify viral genome are used for presumptive diagnosis of the disease. The presence of intracytoplasmic inclusion body known as Bollinger bodies during Histopathological examination is characteristic of the disease. With few exceptions the disease only causes drop in productive performance of birds. Mortality from primary infection of pox virus is uncommon. The presence of vaccine enables to prevent and control the disease easily as there is no effective treatment for the disease.

Key words: Avian Pox · Avipox Virus · Fowl Pox · Skin Lesions · Diphtheritic Form · Birds

INTRODUCTION

Avian pox is a well-recognized infectious disease in birds caused by a family of viruses collectively known as avipoxvirus. Pox virus infections of birds are caused by viruses currently defined by the International Committee on Taxonomy of Viruses as members of a single genus. The Avipox virus genus is one of the eight genera of the sub family Chordopoxviridae; all of which infect vertebrates. Viruses of other seven genera all infect mammals. The Avipox viruses appear incapable of causing disease in mammals; therefore there appears to be no zoonotic potential [1].

FPV is the best studied and prototypic species of the Avipox viruses. There are currently nine other recognized species, yet Avipox virus infections have been observed in more than 230 of the known 900 species of bird, spanning 23 orders. Very little is currently known about the genome diversity, host range and host specificity of the causative agents. These viruses are antigenically and

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immunologically distinguishable from each other, but cross-relationships complicate strain identification and the actual number of species or strains of avian poxvirus are not known. Although fowl-, pigeon-, quail-, canary-, turkey- and juncopox are among some of the identified species, studies in domestic poultry with fowlpox virus (FPV) have been responsible for most of the information currently available. There is evidence of considerable heterogeneity among species of avian poxviruses. Serology has revealed cross-reactivity among several of the viral species, whereas genomic and antigenic characterization has been only moderately useful for identifying differences among strains. A great range of pathogenicity is seen among various species exposed to different types of avian poxvirus. Host specificity appears to be particularly strong in wild birds regarding both pathogenicity and productivity against other strains of the virus [2].

A recently identified condorpox virus isolated from an Andean condor (Vulturgryphus) caused an aggressive, diphtheritic form of the disease in the condor but produced small, localized cutaneous lesions in inoculated chickens, which subsequently received no related cross-protection when challenged with FPV [3].

It is relatively slow-spreading viral disease characterized by skin lesions and/or plaques in the pharynx and affecting chickens, turkeys, pigeons and canaries worldwide. Morbidity is 10-95% and mortality usually low to moderate, 0-50%. Infection occurs through skin abrasions and bites, or by the respiratory route. It is transmitted by birds, fomites and mosquitoes (infected for 6 weeks) [4].

Regardless of the strain of virus or species of bird, the associated pathology is very similar. The most common cutaneous form of avian pox involves the unfeathered parts of the body: legs, feet, face at the base of the beak and eyelids. Lesions consist of epithelial hyperplasia of the epidermis resulting in proliferative, wart like projections. With the diphtheritic form, caseous, necrotic lesions develop in the mucous membranes of the upper respiratory tract, mouth and pharynx. Although very convincing on gross examination, presence of the typical skin lesions is not sufficient to definitively diagnose avian poxvirus infection because nutritional deficiencies or mycotoxins and other agents such as papilloma virus or scaly leg mites could produce similar lesions. Light microscopic evaluation of affected tissues can confirm the presence of the typical large, solid or ring like, eosinophilic intra-cytoplasmic inclusions known as Bollinger bodies. Histopathologic changes include hypertrophy and hyperplasia of epithelial cells resembling a papilloma. Virus isolation and propagation on chorioallantoic membranes of chicken embryo is one method of confirming poxvirus. An alternate means of reaching a confirmatory diagnosis is through electron microscopic (EM) examination of affected tissues [5].

Although the worldwide distribution of avian poxvirus is well known, the effect of poxvirus on populations of wild birds is less well studied. The virus persists in the environment for months. It is more common in males because of their tendency to fight and cause skin damage and where there are biting insects. The duration of the disease is about 14 days on an individual bird basis. This disease occurs in most countries with humid and warm climate. The disease causes depression in growth rate and temporal stop in egg production if layers are infected. Infection with pox virus does not result in primary mortality. Five types of pox viruses cause disease in different species of birds.Turkey pox virus in Turkey, FPV in poultry Canary pox virus in Canaries, Pigeon pox virus in Pigeon and Quail pox virus in Quail [6].

Immune responses to fowlpox virus may be demonstrated by the use of virus neutralization, agar gel immunodiffusion, immunofluorescence, or passive haemagglutination tests, enzyme-linked immunosorbent assay and by immunoblotting [7].

Duration of the disease ranges from 2 to 3 weeks. Mortality percentage is higher in diphtheritic or ocular form than cutaneous form. The main reason of mortality in fowl pox is due to complications such as secondary bacterial infections, blindness or starvation. This disease affects lighter breeds as compared to heavier breeds. Mortality in affected birds may go up to 25%. Poultry birds of age groups are affected with FPV but more common at age of 4-5 months[8].Incubation period (IP) varies from 4-14 days and mortality rate is 50 % [9]. The general objectives of this paper are to overview the general features of the disease condition avianpox; to look at the serological features of avianpox; to show the clinic-pathological features of the disease and to mention some risk factors for the occurrence of the disease.

Etiology: Avian pox is the common name for a mild-to-severe, slow developing disease of birds that is caused by a large, oval, double stranded, brick shaped of about 150-200mu by 265-350mu sized DNA virus belonging to the

genus avipoxvirus in the family Poxviridae. It is enveloped virus which develops in the cytoplasm of infected epithelial cells [10].

Strains of the Virus: This group contains several similar virus strains; some strains have the ability to infect several groups or species of birds but others appear to be species-specific. Yet little is known about the genome diversity and host-range specificity of the causative agents, three common strains have been identified. Cross-relationships complicate strain identification and the actual number of species or strains of avian poxvirus are not known. The three strains are fowl pox virus, pigeon pox virus and canary pox virus. The strains vary in their virulence and have the ability to infect other avian species [1].

Studies in domestic poultry with fowl pox virus have been responsible for most of the information currently available. There is evidence of considerable heterogeneity among species of avian poxviruses. Serology has revealed cross-reactivity among several of the viral species, whereas genomic and antigenic characterizations have been only moderately useful for identifying differences among strains. A great range of pathogenicity is seen among various species exposed to different types of avian poxvirus. Host specificity appears to be particularly strong in wild birds regarding both pathogenicity and productivity against other strains of the virus. A recently identified condorpox virus isolated from an Andean condor (Vulturgryphus) caused an aggressive, diphtheritic form of the disease in the condor but produced small, localized cutaneous lesions in inoculated chickens, which subsequently received no related cross-protection when challenged with fowl pox virus. Regardless of the strain of virus or species of bird, the associated pathology is very similar [2].

Genetic Characterization of Avian pox virus: Vaccines of fowl pox or Pigeonpox virus origin are used by the poultry industry for prevention of fowl pox in chickens and turkeys. In spite of regular vaccination, outbreaks of fowl pox continue to occur in some previously vaccinated chicken flocks. Genetic evaluation reveals integration of full-length Reticuloendotheliasis virus (REV) in the genomes of the majority of field strains of fowl pox virus while only long terminal repeats (LTR) of REV are present in the vaccine strains. Insertion of full length REV in the genome of fowl pox virus has been considered as one the genetic markers of virulence as

Table 1: Virus strains affecting different species of birds.

Virus Strain	Species of bird affected
Turkey poxvirus	Turkey
Fowl poxvirus	Poultry
Canary poxvirus	Canary
Pigeon poxvirus	Pigeon
Quail poxvirus	Quail

Source: Mandal *et al*. [6].

such viruses show enhanced pathogenicity versus those which lack full-length REV insertion. In order to determine whether the outbreaks are associated with the vaccine virus or with a field strain, genetic characterization of new isolates for the presence of REV provirus is important. In this regard, fowl pox virus was isolated from two flocks of chickens that had been vaccinated with fowl pox and/or Pigeonpox virus vaccines. With a view to determine whether full-length REV provirus sequences were present in these isolates, unstained slides of formalin fixed tissue sections were received. DNA was isolated from these tissue sections. Primers that will amplify various size fragments (118, 227 and 419) of REV envelope gene and a fragment of 485 bp fragment of LTR were used. Similarly primers of various sizes (110, 145, 208, 302 and 508) for amplification of A-type inclusion body gene were used. Nucleotide sequences of the amplified products were determined and compared with the published sequences. Additionally, genomic DNA from a Canarypox virus isolated from an outbreak in canaries was evaluated for the presence or absence of any REV sequences [1].

On the basis of polymerase chain reaction analyses, the virus isolated from dried lesions of *C. rufescens*has 80.5% similarity with the virus isolated from *A. berthelotti* and 91.3% similarity with Canarypox, whereas A. berthelotti poxvirus has only 80% similarity with Canarypox. We have two distinct and possibly new avian poxviruses [3].

Vectors as Means of Transmission: Mosquitoes are common mechanical vectors or transmitters of this disease.Culexpipiensand Aedesaegypti are capable of transmitting the disease from infected chickens, as lesions developed from 5 to 10 days after the infected mosquito was allowed to feed on a susceptible chicken. Avian pox is transmitted when a mosquito feeds on an infected bird that has viremia or pox virus circulating in its blood, or when a mosquito feeds on virus-laden secretions seeping from a pox lesion and then feeds on another bird that is susceptible to that strain of virus [3].

Contact with surfaces or exposure to air-borne particles contaminated with poxvirus can also result in infections when virus enters the body through abraded skin or the conjunctiva or the mucous membrane lining that covers the front part of the eyeball and inner surfaces of the eyelids of the eye. Pox virus is unable to penetrate unbroken skin, but small abrasions are sufficient to permit infection [4].

The virus is resistant outside the host remaining viable in dry scabs for long periods but sensitive to heat, lipid, phenol and other disinfectants.The virus produces A-type inclusion bodies in the infected cells. A-type inclusion body protein gene in the genome of fowl pox virus appears to protectthe virus from environmental insults [11].

Epidemiology: Fowl pox is still prevalent in many poultry flocks, because the fowl pox virus can remain alive in four up to ten years, which contaminate the environment. Mosquitoes and other blood-sucking insects can transmit the virus Individuals handling birds at the time of vaccination may carry the virus on their hands and clothes and may unknowingly deposit the virus in the eyes of susceptible birds [12].

Distribution: Avian pox occurs worldwide, but little is known about its prevalence in wild bird populations. The increased frequency of reported cases of this highly visible disease and the involvement of new bird species during recent years suggests that avian pox is an emerging viral disease. Birds can become disease carriers and spread avian pox among local populations, such as between bird feeding stations and along migratory routes used by various bird species. Mosquitoes that feed on birds play the most important role for both disease transmission and long term disease maintenance. However, contamination of perches and other surfaces used by captive birds can perpetuate disease in captivity. Pox outbreaks are commonly reported at aviaries, rehabilitation centers and other places where confinement provides close contact among birds. The disease can spread rapidly when avian pox is introduced into such facilities. Species that would not ordinarily have contact with avian pox virus in the wild often become infected in captivity if the strain of virus present is capable of infecting a broad spectrum of species [4].The disease tends to be seasonal, occurring after mosquito breeding times. It is endemic in Papua New Guinea, where it is significant economically because the only NDV in the country is the non-symptomatic form. It is also a major disease in many other tropical countries [13].

Transmission: Transmission of the avian pox virus can occur in a number of ways. The disease can be spread via mechanical vectors, primarily by species of mosquitoes (at least 10).Research has shown that stable fly may also transmit the disease, suggesting that other blood sucking insects such as gnats can also transmit pox. Transmission occurs when the mosquito feeds on an infected bird that has a viremia (pox virus circulating in the blood) present or on virus-laden secretions from a pox lesion and then feeds on an uninfected bird. Mosquitoes can harbor and transmit the virus for a month or longer after feeding on an infected bird. Experimentally, stable flies have shown the capability of being able to transmit the pox virus [4, 6].

Avian pox can also be transmitted by direct contact between infected and susceptible birds. The virus is transmitted through abraded or broken skin or the conjunctiva (mucous membrane covering the anterior surface of the eyeball). Indirect transmission of the pox virus can also occur via ingestion when food and water sources, feeders, perches, cages, or clothing are contaminated with virus-containing scabs shed from the lesions of an infected bird. The pox virus is highly resistant to drying and may survive months to years in the dried scabs. Indirect transmission can also occur via inhalation of pox virus infected dander, feather debris and air-borne particles. Mosquitoes are probably responsible for transmission within local areas, while wild birds are responsible for outbreaks over greater distances[10-12].Both in poultry and the wild birds on the farms that were heavily infested by fleas there were similarities between viruses in,which fleas may have acted as vectors in transmission of poxvirus [3].

Species Affected: The disease affects a wide variety of bird species around the world. Most, if not all, avian species are susceptible to one or more pox strains. Domestic chickens are highly susceptible to fowl pox, but the disease also affects turkeys and to a less extent ducks, geese, pheasants, quail, canaries and hawks. Mammals are not susceptible to natural infection with avian pox virus. Fowl pox is prevalent where poultry is raised. The disease some-times assume an exceptional virulent form, affecting large numbers of fowls and causing serious losses. It is most prevalent during the fall and winter months, but may

occur at any seasons of the year. Approximately 60 free-living bird species have been reported with avian pox. Most commonly reported in songbirds, upland game birds, marine birds and birds of prey [2].

Risk Factors: Under natural conditions there may be breed differences in susceptibility; chickens with large combs appear to be more affected than those with small combs. The mortality rate is low in healthy flocks but in laying flocks and in chickens in poor condition or under stress the disease may assume serious proportions with mortality rates of 50% or even higher, although such mortality is rare [14]. Organisms can't enter the intact skin. For entry of the virus, injury or broken skin is necessary. Viruses spread through directly. Disease can also spread by other Ectoparasites. Mosquito and flies are known to cause the infections. Infection through the respiratory tract is possible [2-3].

Although wild birds can be infected by pox virus year round, disease outbreaks have been associated with the environmental conditions, the emergence of vector populations and the habits of the species affected. Environmental factors such as temperature, humidity, moisture and protective cover all play a role in the occurrence of this disease by affecting virus survival outside of the bird host. Avian pox virus can withstand considerable dryness, thereby remaining infectious on surfaces or dust particles. Mosquitoes that feed on birds are the most consistent and efficient transmitters of this disease. Mosquito populations are controlled by breeding habitat and annual moisture [15].

The time of appearance and magnitude of vector populations varies from year to year, depending on annual weather conditions. This influences the appearance and severity of the disease in any given year. Only limited studies have been carried out to assess the relations between avian pox and insect vector populations [9].

Bird feeding stations have been the source of numerous poxvirus outbreaks.Contact transmission of the virus through infected surfaces and close association of birds using those feeders is the likely means of transmission during cooler periods of the year when mosquitoes are not a factor and birdfeeders provide additional sources of infection when mosquitoes are present [15].Avian poxviruses are only known to infect birds, so there is no known risk to human or other mammals health from these viruses. Garden birds in the UK, however, may carry other diseases that can affect humans and pets, such as Salmonella, Campylobacter and E. coli bacteria [16].

Clinical Signs: Clinical signs observed with avian pox are weakness, emaciation, difficulty in swallowing and breathing, vision problems, a reduction in egg production, soiled facial feathers, conjunctivitis and edema of the eyelids and the presence of the characteristic wart-like growths on the unfeather portions of the skin and/or formation of a diphtheritic membrane on the upper portion of the digestive tract.In many species, particularly those not in the tit family (e.g. wood pigeon and dunnock), the growths can be relatively mild and may regress with time. Affected birds develop skin lesions but usually appear to feed and move around normally. In some cases (in all species but especially in great tits) the growths can become very large and may impede the ability of birds to see, feed or move around. In these cases the birds become more susceptible to predation and other infections. Whilst the disease in great tits is not invariably fatal and recovery can occur, the condition reduces individual survival, particularly in juvenile birds Avian pox can occur in two forms: cutaneous pox and diphtheritic or "wet" pox [10].

Skin, Dry or Cutaneous Form: Characterized by the appearance of cutaneous eruptions or wart like nodules on the unfeathered parts of fowl, e.g. comb wattle, eyelid, feet, cloaca aperture and under the wings.In young chicks, corner of mouth, nostril and eyelids. Removal of the pox scale (local epithelial hyperplasia) resulted in bleeding.The cutaneous nodules may be very numerous or few in number and they do not necessarily erupt at the same time. At first, the nodules appear as small, whitish foci which rapidly increase in size and become yellowish in color as they develop.In some instances closely adjoining lesions may coalesce and the large developing lesions are rough and gray or dark brown in color. After about 2 weeks of development, the lesions may show area of inflammation at their base and become hemorrhagic. The lesion then undergoes a process of desiccation and scar formation which may last for another week or possible two weeksin uncomplicated cases the process ends with desquamation of the degenerated parts of the epithelial layer. If the desiccated scab is removed in the meantime, a moist sero-purulent exudate is found underneath, covering a bleeding, granulating surface.

When the scab drops off, a smooth scar may be present. The specific process is often modified by the invasion of bacteria which propagate in the degenerated epithelium and may reach the deeper layer of mucous membrane where they catalylze supportive or necrotic processes with the formation of fibrinous deposits [17].

Diphtheritic or Wet form: The type is not as common as the cutaneous form.The eruptions on the mucous membranes are white, opaque, slightly elevated nodules.These processes rapidly increase in size, often coalescing to become a yellowish, cheesy, necrotic material with the appearance of a pseudo membrane. Where these pseudo membranes are removed they leave bleeding erosions. The invasion by contaminated bacterial aggravates the diphtheritic form of the disease. The inflammatory process may extend from the mouth region into the sinuses, particularly the intra orbital sinuses, resulting in a tumor like swelling and may extend into the pharynx, resulting in respiratory disturbance. This form of disease is primarily a problem of young chickens and turkeys[12].

Clinico-pathological Features: Regardless of the strain of virus or species of bird, the associated pathology is very similar. The most common cutaneous form of avian pox involves the unfeathered parts of the body: legs, feet, face at the base of the beak and eyelids. Lesions consist of epithelial hyperplasia of the epidermis resulting in proliferative, wart-like projections. With the diphtheritic form, caseous, necrotic lesions develop in the mucous membranes of the upper respiratory tract, mouth and pharynx [3].

Gross Lesions: Fowlpox may be suspected when skin lesions erupt on various parts of exposed skin (cutaneous form) of affected chickens. A mild form of the disease may remain unnoticed, with only small focal lesions, usually on the comb and wattles. In severe forms of the disease, generalized lesions may occur on any part of the body, such as the comb, wattle, corner of the mouth, around the eyelids, angle of the beak, ventral surface of the wings, legs and vent. Skin lesions may be small and discrete or may involve large areas through the coalescence of adjoining lesions. Coalescence of the lesions around the eyelids can cause complete closure of one or both eyes [11].

The small focal nodules of the skin, initially vesicular, enlarge rapidly because of proliferation of the virus in the epithelium and infiltration by the inflammatory cells. The surface of the lesions is moist for a short time, but it dries soon and develops a rough irregular surface, which becomes yellowish - brown to dark- brown. Removal of such lesions, if they are not completely dry, leaves a hemorrhagic moist surface. When the scab is dry, however, it drops off, leaving a scar. Often the virus also affects the mucous membrane of the mouth, nares, pharynx, larynx, esophagus and trachea, causing white or opaque eruptions which coalesce and expand rapidly, later becoming ulcerated and covered with a yellowish caseous necrotic exudate. Mucous membranes of mouth, larynx, pharynx and trachea (diphtheritic form) undergoing the extensive fibrino-necrotic process develop a diphtheritic membrane. A hemorrhagic surface is left when the diphtheritic membrane is removed. Lesions in the mouth, tongue and esophagus interfere with the feeding and lesions of the trachea often result in the formation of tracheal plugs. In such cases, there is serious difficulty in respiration, with signs of gasping and suffocation may result. This form of the disease may simulate signs of laryngotracheitis[4].

The cutaneous form is the most commonly observed and is a self-limiting infection with the lesions regressing and forming scars. Initially, this form of pox appears as a small white, pink or yellow vesicle (blister) on un feathered parts of the skin (feet, legs, base of the beak, eye margins and head). The vesicle is a result of the separation of the surface layer of the skin with the formation of pockets of watery fluid rich in multiplying virus. The vesicles become nodules as they increase in size, coalesce and burst. Lymph from the cells congeals and scabs are formed. The surface of the nodules become rough and dry and the color changes to dark brown or black. The size and number of nodules present depends on the stage and severity of the infection. Bacteria may gain access causing secondary infection and resulting in a purulent discharge (pus) and necrosis. Eventually, the scab falls off and a scar forms at the site. It takes 2 to 4 weeks for complete healing of the affected areas on the skin providing the lesions aren't too extensive thereby preventing the bird from feeding [12].

The diphtheritic form involves the mouth, throat, trachea and lungs and consists of yellow or white, moderately raised, moist cheese-like necrotic areas. A diphtheritic membrane forms and may restrict air intake and result in labored breathing and possible suffocation.This form of avian pox probably occurs more frequently in wild birds than it is reported because it is less observable than the cutaneous form. Also, the more severe consequences of wet pox undoubtedly cause

greater morbidity and mortality, thereby leading to removal of infected birds by predators and scavengers.Splenomegaly, possibly in response to bacterial infection may be observed [5].

Microscopic Lesions: Histologically, intracytoplasmic inclusion bodies (Bollinger bodies) are present in the infected skin and respiratory tract mucosa. In the diphtheritic form of the disease, nodular hyperplasia (increase in the number of cells) of the mucosa is observed.Histopathological examination of cutaneous lesions from 17 great tits detected lesions characteristic of avian poxvirus infection: severe hyperplasia and ballooning of epidermal cells; multiple coalescing foci of necrosis and eosinophilic, intracytoplasmic inclusions characteristic of Bollinger bodies. The central cream- or yellow-coloured core of each lesion examined consisted of amorphous, acellularproteinaceous material (likely necrotic tissue). Electron microscopic examination of diseased tissues from two birds detected multiple intracytoplasmicvirions with characteristic avipoxvirus morphology [3].

Smears of materials scraped from cutaneous lesions or post mortem secretions from diphtheritic lesions (or airway epithelium in the case of pneumonia) can be stained with HE or Gimenez. The presence of eosinophilic inclusion bodies (A-type inclusions, Bollinger bodies) in the cytoplasm is diagnostic of poxviruses. Basophilic or B-type inclusion bodies may also be seen in the cytoplasm (these represent the sites of virus replication, the so called 'viral factories') as may Borrel bodies (the virions themselves) [2].

Microscopic examination of the nodular lesions from the junco revealed a densely hyperplastic epithelium with almost every cell at numerous foci containing a large pink-staining cytoplasmic inclusion pathognomonic of infection with avian pox viruses. Individual cells were swollen and rounded but not detached from each other. Cytoplasmic inclusions were globular, like those of fowlpox, but often seemed less compact. In addition many infected cells contained well-defined nuclear inclusions which appeared as acidophilic, smooth, compact, usually oval bodies lying in a clear nuclear space. Nuclear chromatin stained densely against the nuclear wall and nucleoli were demonstrable. This pathologic appearance differed in at least one respect from that of other known avian pox diseases; the following observations on the behavior of the virus isolated from this lesion describe in some measure its pathogenic nature and help to define it as an entity [18-20].

Diagnosis: A presumptive diagnosis of avian pox can be made due to the gross lesions on the body. Confirmation of avian pox is accomplished by microscopic examination for the characteristic Bollinger bodies. Virus isolation by transmission of the organism via egg inoculation, serological results and polymerase chain reaction can also be a means of confirming the disease [21-22].

Identification of the Agent: Fowl pox virus multiplies in the cytoplasm of epithelial cells with the formation of large intracytoplasmic inclusion bodies (Bollinger bodies) that contain smaller elementary bodies (Borrel bodies). The inclusions can be demonstrated in sections of cutaneous and diphtheritic lesions by the use of haematoxylin and eosin (HE), acridine orange or Giemsa stains. The elementary bodies can be detected in smears from lesions, for example by the Gimenez method, which is described below. Electron microscopy can be used to demonstrate viral particles of typical poxvirus morphology by negative staining or in ultrathin sections of infected tissues [7].

A smear technique for fowlpox; Place a drop of distilled water and the lesion (cutaneous or diphtheritic) on a clean slide. Prepare a thin smear by pressing the lesion with another clean slide and rotating the upper slide several times. Air dry and gently fix the smear over a flame. Stain the smear for 5-10 minutes with freshly prepared primary stain (8 ml stock solution of basic fuchsin mixed with 10 ml of phosphate buffer, pH 7.5 and filtered through Whatman filter paper Wash thoroughly with tap water. Counterstain with malachite green (0.8% in distilled water) for 30-60 seconds. Wash the smear with tap water and then dry. Examine the smear under oil immersion. The elementary bodies appear red and are approximately 0.2-0.3 μm in size [7, 21].

Virus Isolation: Fowlpox virus can be isolated by the inoculation of suspected material into embryonated chicken eggs. Approximately 0.1 ml of tissue suspension of skin or diphtheritic lesions, with the appropriate concentration of antibiotics, is inoculated on to the chorioallantoic membranes (CAMs) of 9-12-day-old developing chicken embryos. These are incubated at 37°C for 5-7 days and then examined for focal white pock lesions or generalized thickening of the CAMs. Histopathological examination of the CAM lesions will reveal eosinophilicintracytoplasmic inclusion bodies following staining with HE. Primary chicken embryo fibroblasts, chicken embryo kidney cells, chicken embryo dermis cells, or the permanent quail cell line QT-35, can

also be used to propagate fowlpoxvirus. The adaptation of virus strains to cell cultures is an important requirement for plaque formation, as not all strains will form plaques initially [10].

Serological Tests: Serologic tests such as the AGID are helpful for thoroughly studied poxviruses of domestic species, but the test suffers from limitations. Because of cross-reactions, one cannot differentiate different species or strains of poxvirus. Yet, lack of cross-reaction with other strains of the virus yields negative results, despite histo-pathologic evidence of avian poxvirus infection, as was the situation in this case. The diagnostic AGID assay recognizes both clearly and weakly positive samples, presumably because of the unpredictable cross-reactivity among different strains of the various identified avipoxviruses. The diagnostic agar gel immunodiffusion (AGID) technique was used to determine the presence of cross-reacting antibodies to avian poxvirus in the sera from birds from the Canary Islands. This diagnostic assay is designed to detect avian poxviruses that cross-react with FPV and pigeon pox virus strains that commonly affect domestic birds.positive, Because of the conservative nature of this technique, reactions are classified into three categories of response: weakly positive, or negative[3].

Although both cell-mediated immunity (CMI) and humeral immunity play an important role in poxvirus infections, routine use of the CMI test is not convenient. Therefore, serological tests, such as virus neutralization (VN), agar gel immunodiffusion (AGID), passive haemagglutination and fluorescent antibody tests as well as the enzyme linked immunosorbent assay (ELISA), are used to measure specific humeral antibody responses. Evidence of successful immunization with vaccine can be determined by examining a flock 7-10 days after vaccination for 'takes'. A take consists of a swelling of the skin or a scab at the site where the vaccine was applied and its presence is evidence of successful immunization. Serology for antibodies to avian poxvirus was unrewarding, yielding only three positives of 128 serologic samples, of which 52 birds had physical evidence of pox infection [3,20].

Monoclonal antibodies against the three major immunodominant structural antigens of FWPV have been isolated and characterized. One target is the 39k immunodominant core protein equivalent to the vaccinia virus A4 protein. Variability is observed in the size of this protein between different isolates and strains, because of diagnostic differences in the number of repeats of a 12 amino acid motif. Another target is the 35k protein equivalent to VACV H3, found on the surface of the infectious, intracellular form of the virus, as is the third target the 63k P4C protein, responsible for virions entry into A-type inclusion bodies. None of these monoclonal antibodies appears to be neutralizing [2, 6].

Avian poxvirus strains from one host can provide reciprocal immunity to other host species and cross immunity has been proven for several strains of avian pox. For example, chickens may be vaccinated with live pigeon pox strains because they stimulate immunity to typical strains of avian poxviruses without causing serious disease. It seems probable that immunity to avian pox exists in a spectrum of continuous adaptation to various avian host species [11].

Antigenic variations r between strains of fowl pox virus can be evaluated by means of immune-blotting or Western Blotting. In this method, viral antigens separated by SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) are reacted either with polyclonal or monoclonal antibodies against fowl pox virus. This method is not convenient for routine diagnosis [7].Evidence for prior infection or for ongoing infection in the flocks, can be determined by serological methods. The older technique of gel precipitation has essentially been suspended by ELISA, as used in commercial flock monitoring kits. VN tests are more specific but are technically more demanding [2,8].

Molecular Methods: Restriction fragment length polymorphism (RFLP) analysis can be used for comparison of field isolates and vaccine strains of fowl pox virus. However, this procedure is not used in routine diagnosis. Cloned genomic fragments of fowl pox virus can be used effectively as nucleic acid probes for diagnosis of fowl pox. Viral DNA isolated from lesions can be detected by hybridization either with radioactively or non-radioactively labeled genomic probes. This method is especially useful for differentiation of fowl pox from infectious laryngotracheitis when tracheal lesions are present. Genomic DNA sequences of various sizes can be amplified by the polymerase chain reaction (PCR) using specific primers. This technique is useful when there is only an extremely small amount of viral DNA in the sample [10].

Impact on Health of Birds

Development of the Disease: Virus enters a skin cell and then spreads from cell to cell locally. Some virus enters the blood to cause a blood infection (viraemia).Although

there is spread to internal organs, no changes are seen.However, it is likely that there is some viral growth in certain organs such as the liver and spleen and a secondary viremia occurs. Virus can also enter again into the skin cells and a generalized disease can occur, although this is rare [12].

Avian pox was considered to be a significant contributory factor to the cause of death for all birds examined post mortem. Injuries from predator attacks are likely the ultimate cause of death. The periocular location of skin lesions in many cases, coupled with the large lesion size, meant that the affected eye was frequently completely obscured; affected birds would therefore have severely compromised vision and would have been vulnerable to predator attack. Large skin lesions on the wing of some birds were considered likely to have interfered with flight and therefore predator avoidance. The birds with splenomegaly may have had a bacteremia secondary to pox which, if present, could have predisposed the birds to predation [19].

The most common form is cutaneous and it consists of warty nodules that develop on the featherless parts of the bird. This form of the disease is usually self-limiting; the lesions regress and leave minor scars. However, these nodules can become enlarged and clustered, thus causing sight and breathing impairment and feeding difficulty. Secondary bacterial and other infections are common with this form of the disease and these infections can contribute to bird mortality. In some birds, feeding habits result in the large warty nodules becoming abraded and then infected by bacterial and fungal infections. When the virus is transmitted from one bird to another through direct contact, such as pecking, or through mosquito bites, the resulting clinical expression is the dry form of the disease. In the dry form, Fowl Pox is characterized by the presence of specific lesions on the skin. These vary from raised, reddened lesions through a pustular form to a dry, scabbed lesion. The clinical effect on the flock with this form of the disease is minimal, but feed consumption and production can be slightly lowered. The wet form is more severe clinically, causing interference with eating or breathing and resulting in death due to asphyxiation when the trachea is affected[12, 22].

CONCLUSION AND RECOMMENDATION

Avian pox is an important viral disease that has been described since the middle of the 19th century which affecting a wide range of bids worldwide. It is a relatively slow spreading disease causing different effects on birds. The primary effect is that it predisposes birds to secondary diseases by giving access for the introduction of pathogens and weakening the immunity. The prevalence of the disease is high in backyard chicken production than intensive system which makes it an economically important disease in countries like Ethiopia in which around 98% of poultry production system is backyard. The backyard chickens are more exposed to the disease because they are in close association with the mosquitoes and their environment is mostly contaminated. The major economic importance of the disease is that mortality mainly due to secondary infections and reduction in the productive performance of the birds. Because the genomic sequences of only a small number of isolates are known, determining the species of poxvirus involved with naturally occurring disease remains elusive. It will be essential to investigate environmental, immunologic and other biological factors contributing to the remarkable, species-specific susceptibility to poxvirus in these isolated populations to predict the effect on population stability and to reverse this alarming trend in disease occurrence.

Managemental practices should be implemented in poultry production areas to prevent the incidence of the disease by improving the hygiene of birds. Vector transmission should be prevented by controlling mosquitoes.A well programmed vaccination should be practiced to develop the immunity of birds andthorough scientific investigations should be done especially on wild strains of avian pox viruses as much of the information on avian pox viruses is limited in poultry.

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